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Morphological variation of *Medicago sativa* subsp. *falcata* genotypes and their hybrid progeny

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Summary

Semi-hybrid alfalfa cultivars offer the possibility of capturing non-additive genetic variation. *Medicago sativa* subsp. *falcata* and subsp. *sativa* have been shown to form a heterotic pattern for biomass yield. Objectives of this study were to examine morphological variation in a broad range of falcata germplasm and to determine how falcata morphological variation *per se* is related to the performance of falcata germplasm in hybrid crosses with subsp. *sativa*. Falcata genotypes from 40 populations spanning the subspecies native range were selected and biomass yield, plant width, plant height, growth angle, biomass density, plant maturity, and regrowth after cutting were measured on the genotypes and their hybrid progeny three times throughout the growing season. In addition weekly plant heights were measured and growth rates were determined with a Gompertz function. Falcata parental genotypes exhibited a full range of phenotypes for plant width, plant height, growth angle, density, and maturity. Heterosis was not only observed for biomass yield but also for plant width, plant height, and more erect growth habit. The top yielding sativa-falcata hybrids had increased plant width, plant height, and plant density. European germplasm was taller and had faster regrowth than Asian material. Sativa-falcata hybrids produced biomass yield superior to the mid-subspecies mean only after two to three weeks of growth prior to first and third harvests. Prior to second harvest, biomass production was inferior to the mid-subspecies mean for 30 days. Hybrids using falcata as one parent are not currently adapted to intensive harvest management due to their slower regrowth.

Abbreviations: FC – Falcata clones; SC – Sativa clones; SFC – Sativa by falcata crosses; SSC – Sativa by sativa crosses

Introduction

Hybrid breeding methods could possibly increase biomass yield in alfalfa (Brummer, 1999). Effective implementation of a hybrid breeding system would be optimized by improving at least two independent and complementary populations, which combine well to produce heterosis. *Medicago sativa* subsp. *falcata* (hereafter 'falcata') has been identified as a general source of germplasm populations that show heterosis in crosses with elite *Medicago sativa* subsp. *sativa* (hereafter 'sativa') breeding material (Riday & Brummer, 2002ab; Riday et al., 2002). Unfortunately, few improved falcata breeding populations exist, making implementation of a sativa-falcata hybrid breeding system useful for cultivar development difficult at this time.

Geographically, falcata are distributed in the colder areas of Russia, Mongolia, Scandinavia and China (Hansen, 1909; Lesins & Lesins, 1979). The slower regrowth, early onset of autumn dormancy, and decumbent growth habit of falcata germplasm are undesirable traits for an intensive agricultural system (Lesins & Lesins, 1979; Riday & Brummer, 2002b). One of our breeding objectives is to identify falcata germplasm that has agronomic characteristics similar to elite sativa populations and that also shows biomass yield heterosis in crosses with upper Midwestern US sativa cultivars.

The underlying causes of the sativa-falcata heterotic pattern for biomass yield are obscure but understanding them may aid selection programs. Traits such as growth habit, growth rate, and growth form are likely involved, and determining how they interact to produce heterosis may allow dissection of the heterotic pattern for biomass yield. We have previously identified the divergence of height, maturity, and regrowth between falcata and sativa genotypes as being associated with specific combining ability for biomass yield (Riday et al., 2003). Sativa-falcata hybrids exhibited negative heterosis for the height of regrowth during the midseason and autumn; however, at biomass harvest, we observed positive heterosis for height (Riday & Brummer, 2002b). Obviously, then, at a given point prior to harvest, sativa-falcata hybrids transition from negative to positive height heterosis. If the transition to positive heterosis for height happens much later than 30 days after harvest, then sativa-falcata hybrids would be at a disadvantage under an intensive harvesting regime. Further, our visual observations, quantified with a vegetative density score, suggested that sativa-falcata hybrids show a heterotic advantage for vegetative density (Riday & Brummer, 2002a). A more quantitative measurement of vegetative density than a score may enable a robust determination of its importance for biomass yield and yield heterosis.

Our previous experiment (Riday & Brummer, 2002a) used a small set of genotypes, so the universality of the relationships between morphology and yield heterosis can not be conclusively discussed without evaluating a broader set of germplasm. Currently, the National Plant Germplasm System's *Medicago* collection contains 470 falcata accessions (USDA, GRIN, 2003). Based on a broad sampling of this germplasm, Riday & Brummer (2003) determined that the best performing sativa-falcata hybrids were derived from western Eurasian falcata genotypes crossed to elite sativa germplasm.

The objective of this study was to assess the morphological performance of these western Eurasian genotypes *per se* and in testcrosses with elite sativa germplasm to identify agronomic strengths and weaknesses for future selection objectives. Due to higher yields of European germplasm, we hypothesize superior agronomic performance of this germplasm.

Materials and methods

Plant materials

A total of 125 genotypes was used as parents in this experiment (Table 1, Figure 1). Sixteen were elite sativa genotypes from four populations, designated as testers: five genotypes from Pioneer Hi-bred International (Des Moines, IA), three genotypes from Forage Genetics (West Salem, WI), three elite genotypes derived from Hungarian germplasm also from Forage Genetics, and five 'Innovator +Z' genotypes derived from one generation of inbreeding. Four wild sativa genotypes from different populations were also included. The remaining 105 genotypes were falcata, derived from 37 wild or semi-improved populations from throughout the native range of falcata. Twelve of 37 falcata populations and one of four sativa populations had variegated flowers suggesting past sativa-falcata introgression (Table 1). Unless noted otherwise, genotypes from variegated populations were designated as falcata. In several populations during the autumn of 1999, vigorous genotypes were visually selected from a space planted germplasm evaluation trial at Ames, IA (Brummer et al., 1997). The trial was in its second post-establishment year and had been harvested twice during 1999. At the time of selection, most plants were dormant with minimal regrowth, so our selections were those with less dormancy than the overall populations. The most vigorous two to three individuals from within the given population were selected.

All 125 genotypes were crossed to the four tester populations in the greenhouse during the autumn/winter 1999 to 2000, producing a total of 500 cross entries, 76 of which were sativa by tester and 424 falcata by tester. Single plant-to-plant crosses were made between test genotypes and individual plants in the tester populations; florets were not emasculated. Usually the tester was used as the female, due to their higher seed production. Several falcata genotypes exhibited low pollen production; in those cases, they was used as the female. For most testcross entries, no self-fertilization was observed based on flower color segregation, although a few entries expressed self-fertilization up to $\sim$10%. The self-fertilization always occurred when the falcata parent used as male was agronomically 'weak.' For field measurements other than biomass yield, obvious self-fertilized individuals were avoided when possible.

In spring 2000, seed from the 500 cross entries; the three wild sativa and 37 wild/semi-improved falc-

Table 1. Origin of the 44 populations evaluated and number of genotypes selected from 44 populations used in this study

Population	Origin[†]	No.[‡]	Population	Origin	No.
			Wild Germplasm		
1. PI 631591	9°38', 47°15'	2	16. PI 631612	84°00', 29°00'	3
2. PI 251836[§]	11°19', 44°10'	1	17. PI 631601	84°38', 44°09'	4
3. PI 631579[¶]	13°29', 45°52'	3	18. PI 538993[#]	85°53', 50°50'	3
4. PI 253451[§¶]	16°38', 46°22'	1	19. PI 577561	85°59', 52°00'	4
5. PI 631796	16°38', 49°12'	5	20. PI 499661	88°31', 44°05'	2
6. 5291/88[††]	17°52', 47°12'	1	21. PI 631645	102°34', 47°23'	5
7. 5299/88[¶††]	17°55', 47°13'	1	22. PI 631639	112°00', 47°30'	3
8. PI 631857	18°37', 57°17'	4	23. PI 499548	116°02', 43°58'	1
9. PI 494661[¶#]	23°25', 46°55'	5	24. W6 16608	118°06', 48°00'	1
10. PI 494658[§]	26°54', 46°34'	1	25. PI 214218[¶]	Denmark	1
11. PI 502441[#]	43°53', 46°11'	5	26. PI 631806[#]	North Russia	4
12. PI 384890[§]	55°01', 36°25'	1	27. PI 314092[¶#]	South Russia	4
13. PI 538985[#]	56°19', 50°50'	5	28. PI 631811[‡‡]	S. Kazakhstan Pro.	1
14. PI 440539[¶]	76°57', 43°15'	1	29. PI 573175[#]	China	3
15. PI 631608	84°00', 29°00'	3			
			Improved Germplasm		
30. PI 631620[¶]	77°00', 34°00'	2	36. IA-3018[#]	Ames, IA	5
31. PI 631597[#]	45°55', 51°30'	5	37. Lodgeland[¶]	Brookings, SD	2
32. PI 631596[¶]	91°48', 53°27'	2	38. SD 201[‡‡]	Brookings, SD	1
33. PI 502453[#]	Russia	4	39. PI560333 (WISFAL)[#]	Madison, WI	5
34. PI 631797[¶]	Russia	3	40. PI 468015[¶]	–107°48', 51°25'	1
35. PI 631799[¶]	Latvia	2			
			Elite Sativa Germplasm		
41. Pioneer Hi-Bred	Johnston, IA	5	43. Hungarian	West Salem, WI	3
42. Forage Genetics	West Salem, WI	3	44. ABI Alfalfa	Napier, IA	5

[†] Longitude, Latitude.
[‡] Number of genotypes per population used for crossing.
[§] Wild sativa population.
[¶] Populations that contain some genotypes with variegated flowers.
[#] Populations from which genotypes were both visually and randomly selected.
[††] Accession from Institute of Agrobotany, Hungary.
[‡‡] Colchicine doubled diploid.

ata populations from which the genotypes used for crossing were derived, and two check cultivars (Vernal and 5454) were planted in the greenhouse. Stem cuttings of the 125 parental genotypes were made at the same time. A total of 667 entries was included in this experiment (500 crosses, 125 parental clones, 40 populations, and 2 checks).

Experimental design

Seedlings and cuttings were hand transplanted at the Agronomy and Agricultural Engineering Research Farm west of Ames, IA in a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) on 1 Aug 2000 and at the Northeast Research Farm south of Nashua, IA in a Readlyn loam (fine-loamy, mixed, mesic Aquic Hapludolls) on 8 Aug 2000. At each location, the field experiment was arranged in an augmented plot design consisting of 20 incomplete blocks of 40 plots each, for a total of 800 plots. All 665 entries were present once, except for 55 randomly selected entries that were replicated twice. These 720 entries were then distributed among the incomplete blocks. In addition, each incomplete block contained the two check cultivars and was completed by the addition of replications of two or three entries not already present in that incomplete block. Each plot consisted of sixteen plants that were planted in a two by eight

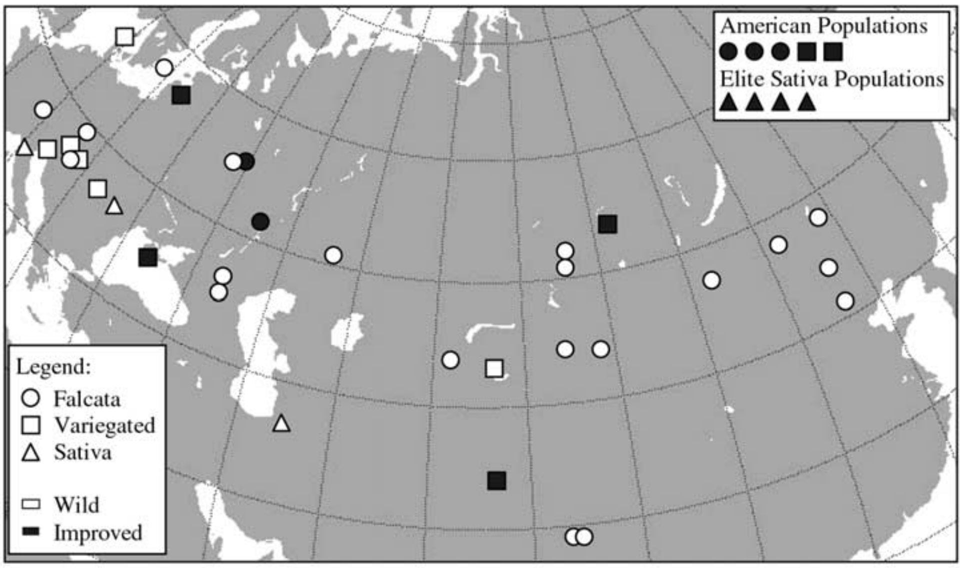

Figure 1. Geographical distribution of alfalfa populations selected for evaluation and their location of origin.

plant grid, with plants separated by 30 cm within a plot and plots separated by 75 cm on all sides.

Harvests for biomass yield were taken on 3 June 2001, 24 July 2001, 11 Sep 2001, 30 May 2002, 13 July 2002, and 30 Aug 2002 in Ames and on 10 June 2001, 18 July 2001, 30 Aug 2001, 13 June 2002, 18 July 2002, and 12 Sep 2002 in Nashua. Biomass yield and biomass yield heterosis were calculated for each harvest (Riday & Brummer, 2003).

Concurrent with biomass harvests (no more than three days prior to harvest), maturity, plant width, and plant height were measured on each plant. Maturity was visually scored on a 1 = early vegetative to 9 = ripe seed pod scale (Kalu & Fick, 1981). Plant width was measured on each plant in a plot from the center of the crown to the furthest horizontal natural growth point and averaged for a single plot value. Similarly, plant height was the average natural height of all plants in the plot. In addition to measurements concurrent with harvest, plant height was also measured on every plot approximately once per week from plant emergence in the spring until the first damaging frost in the autumn.

Vegetative density and growth angle were derived from measurements taken at the time of each harvest. Vegetative density was calculated as the dry matter mass of sixteen plants (i.e., the full plot, or sixteen times the g plant^{-1} estimate) divided by the volume of the vegetative matter in the plot. Vegetative matter volume was estimated based on a 3-dimensional space, similar in shape to a French fry or bratwurst paper dish, generated from plant height, width, and plot layout (Figure 2). Growth angle was calculated as the arctangent of the plant height divided by the plant width.

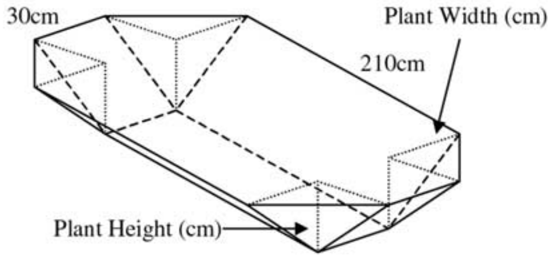

Figure 2. Diagram of plot shape used to estimate plot volume. Plot shape based on transplanting layout and plant with and height at harvest.

Data analysis

Experiment wide entry means

Experiment wide least square means were calculated for plant width, plant height at cut, plant growth angle, vegetative density, and maturity for each harvest. The MIXED procedure of the SAS statistical software package was used with years and entries treated as fixed effects and locations and incomplete blocks designated as random effects (Littell et al., 1996). Homogeneous variances were assumed among different entries. Biomass yield and biomass yield heterosis on a total yearly and harvest basis were calculated as described in Riday & Brummer (2003). Since each genotype was crossed to four testers, a genotype by tester average, on an experiment wide basis was obtained for each genotype used. Thus, for each trait 250 means are reported (106 falcata clones [FC], 19 sativa clones [SC], 106 sativa × falcata crosses [SFC], and 19 sativa × sativa crosses [SSC]), and hence, was represented by 250 mean values. Height Series Data

We wanted to be able to compare the height over time of SFC, SSC, FC, and SC based on a single set of data averaged across both locations and both years. We had collected height data on roughly weekly intervals throughout the growing season for all plots, but given the size of the experiment, all plots at a given location could not always be measured on the same day. Additionally, the dates of harvest varied across locations and years. These variations complicated the assembling of a data set that could be averaged across all four environments (i.e., location-year combinations). Therefore, we adjusted individual plot data as described below so that each environment could be assimilated into a common data set that would allow us to calculate average growth curves for each of four growth periods (i.e., prior to first harvest, between first and second harvest, between second and third harvest, and after third harvest). After we made these adjustments for environments, we could then develop a single height growth curve for each entry for use in subsequent analyses.

We aligned height measurements taken in the four different environments based on the number of days after harvest on which a given measurement was taken. Next, least squared means were computed within each environment for those entries measured on a given day, to adjust for within environment blocking effects. After having removed incomplete block effects, a mean value was calculated for each day from all entries measured on that day. A Gompertz curve (Gompertz, 1825) (height = $\mathrm{C}e^{-e^{-\mathrm{B(Day-M)}}}$), where C = upper asymptote, M = time of maximum growth rate, and B = growth rate) was then fit through these average height values within each of the four environments (i.e., Ames 2001, Ames 2002, Nashua 2001, and Nashua 2002) for each growth period.

An experiment wide Gompertz growth curve was estimated from the data across all four environments. This curve was then compared with the curve obtained from each individual environment, and the deviation of the location specific curve from the experiment wide curve was estimated. The location mean value for each entry measured on a particular day was then adjusted based on this deviation, resulting in a data set for which environmental effects were removed. For each day, these data were averaged by entry across environments to produce only one height value for each entry for any given day within each growth period. At the end of this process, each entry was represented by a single value on each day it was measured, and that value was adjusted for mean environmental effects and for the effects of incomplete blocks within an environment. These data of each individual entry were used to compare SFC, FFC, FC, and SC.

Regrowth and autumn height

Regrowth for each growth period was estimated for each entry by averaging daily height measurements for the first 20 days after spring emergence and each harvest. Similarly, autumn asymptotic height was estimated as the average of daily height measurements from days 33 to 52 post third harvest (i.e., the final 20 days of the autumn height series data). Because plant height was not measured on every entry on the same day as described above, the missing days were interpolated for the regrowth calculation. Interpolating missing daily values allowed regrowth comparisons between regrowth periods. Using the experiment wide height series data for each entry generated from the adjustment procedure detailed above, we estimated the missing height data with a linear model fit between the available measurement dates. This height interpolation was only done to calculate regrowth and was not used for growth curve analysis below.

Time of maximum growth rate and growth curves

Gompertz growth curves (Gompertz, 1825) were fit through height series measurements using PROC NLIN (SAS, 2000). Height series data were based on averages height data across entries within the four analyzed categories (i.e., SSC, SFC, SC, and FC). During the first growth period, the lower asymptote was fixed at zero cm. The lower asymptotes for subsequent growth periods were fixed at five cm, consistent with the cutting height. The time of maximum growth rate (the M component of the Gompertz curve) or the number of days after harvest at which maximum growth rate was achieved was derived directly from the fitted Gompertz curve.

Mean comparisons and other analysis

All mean comparisons among groupings of entries for a specific trait were based on experiment wide mean data sets. Comparisons were done using t-tests between various groupings of the data. Least significant differences were approximated by averaging all pairwise standard error values generated from the DIFF option of the LSMEANS statement of PROC MIXED and multiplying this value by the appropriate t-value (SAS, 2000). Unless otherwise noted, all significance levels are reported at the 0.05 level.

Results

Subspecies mean comparisons

The sativa × falcata crosses had greater plant width than SSC, FC, or SC during all three harvests (Table 2). Falcata clones were wider than SSC and SC at the first and third harvests, but similar at second harvest. For growth angle, SSC were equivalent to SC and always more erect than SFC; FC were the most decumbent class (Table 2). No differences were observed among classes for vegetative density during the first harvest, but during second harvest, FC were more dense than all other classes, which were equivalent. At third harvest, FC had the highest vegetative density, SFC and SSC were equivalent, and SFC were more dense than SC (Table 2). Falcata clones matured more slowly than the other three classes at all three harvests. The SFC had similar maturity as SSC and SC at first harvest, but later maturity at harvests two and three (Table 2). During spring, the parental clones recovered more slowly than crosses, which may reflect a ‘cutting’ versus ‘seedling’ effect. At the three succeeding regrowth periods, SSC and SC had the fastest regrowth, SFC were intermediate, and FC were slowest (Table 2). These results are congruent with our previous experiment were we noted positive mid-subspecies heterosis for height, but negative heterosis for regrowth during and after harvests (Riday & Brummer, 2002b).

The day during the spring and autumn regrowth period at which the maximum growth rate was attained was equivalent for all classes. However, during second and third growth periods, or after first and second harvest, respectively, SSC and SC reached maximum growth rate the fastest. After first harvest, the FC didn’t reach their maximum growth rate until 13 days later than the other classes; SFC and FC were the slowest to reach maximum growth rate after second harvest (Table 2). Finally, plant height at harvest consistently showed SSC to be taller than FC with SFC intermediate (Table 2).

Mid-subspecies heterosis

Sativa × falcata crosses were compared to the mean of SSC and FC to determine mid subspecies deviations for the seven traits measured (Table 2). Plant width and growth angle, measured at harvest, showed positive deviations for all harvests, except growth angle at third harvest (Table 2). No deviation was seen for vegetative density or for time of maximum growth. Maturity showed a positive deviation only during first harvest. Positive deviations were observed for spring regrowth and first through third harvest plant height. In contrast, second through fourth growth period regrowth and autumn height showed negative mid-subspecies deviations (Table 2).

Mean comparisons between falcata groupings

In order to focus future falcata germplasm collection and improvement efforts, we attempted to identify particular morphological traits associated with particular subsets of falcata genotypes. Therefore, we split the FC into the following groups: (i) genotypes producing the top 20% yielding SFC versus the remaining 80%, (ii) selected versus random genotypes from populations in which selection had been practiced, and (iii) genotypes from wild European versus Asian populations (Table 2). We then compared the three falcata groupings among the SFC progeny of the genotypes and among the clones *per se* (i.e., the FC). We have previously shown that the selected and European genotypes were strong indicators of increased hybrid yield and heterosis (Riday & Brummer, 2003).

Comparisons among sativa × falcata crosses

Comparisons between the top 20% yielding SFC and the remainder showed that the top yielding hybrids had greater plant width and vegetative density for each harvest (Table 2). Top yielding SFC also had increased regrowth after second and third harvest and increased plant height at the third harvest (Table 2). Selected genotypes had superior SFC progeny only for third and fourth growth period regrowth compared to randomly chosen genotypes from the same population (Table 2). This is not surprising since the selected genotypes were from an autumn visual selection for plant

Table 2. Entry class (sativa × sativa and sativa × falcata crosses; sativa and falcata clones) and falcata genotype grouping (Top 20% yielding SFC vs. remainder; within the same population selected vs. unselected genotypes; and wild European vs. wild Asian populations) comparisons for traits measured at (i) three harvest: plant width, plant growth angle, vegetative density, and maturity score; and (ii) traits measured during four growth periods: regrowth, time of maximum growth, and plant height at h arvest. Reported means are based on two Iowa locations during 2001 and 2002

Entry Class	Width			Growth ang.			Veg. density			Maturity			Regrowth				Maximum growth				Plant height			
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	Spr.†	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	Aut.‡
	— cm —			— deg° —			— mg/cm^{-3} —			— score —			— cm —				— day —				— cm —			
Sativa × Sativa Crosses	31c§	30b	25c	61a	63a	63a	1.2a	1.6b	1.2bc	4.5a	6.4a	5.0a	0.39a	19a	18a	16a	56a	13ab	14a	13a	67a	59a	55a	45a
Sativa × Falcata Crosses	35a	32a	30a	53b	58b	54b	1.3a	1.7b	1.4b	4.4a	6.1b	4.6b	0.43a	12b	12b	9b	55a	19abc	18b	14a	60b	51b	44b	22b
Sativa Clones	29c	29b	25c	58a	61ab	61a	1.3a	1.7b	1.2c	4.3a	6.3a	4.9a	0.23b	19a	17a	16a	58a	12a	14a	12a	59b	54ab	51a	43a
Falcata Clones	33b	30b	28b	36c	46c	39c	1.3a	1.9a	1.6a	4.1b	5.9c	4.3c	0.20b	9c	9c	7c	68a	32c	17ab	12a	37c	33c	26c	11c
Mid SSC-FC Deviation¶	4***	2**	3***	5**	3**	3^{NS}	0.0^{NS}	–0.1^{NS}	0.0^{NS}	0.2*	0.0^{NS}	0.0^{NS}	0.13***	–2***	–1***	–2***	–6^{NS}	–2^{NS}	2^{NS}	1^{NS}	8***	5***	3*	–6***
Falcata Groupings																								
Sativa × Falcata Crosses																								
Top 20 Yielding Gen.	37*	33*	31**	53^{NS}	58^{NS}	54^{NS}	1.5***	1.8***	1.5***	4.4^{NS}	6.1^{NS}	4.6^{NS}	0.44^{NS}	13^{NS}	13**	10*	53a	19a	17a	14a	62^{NS}	53^{NS}	47*	25^{NS}
Selected Gen.	35a	32a	29a	54a	59a	55a	1.5a	1.7a	1.5a	4.4a	6.1a	4.6a	0.42a	13a	13a	10a	54a	18a	17a	14a	61a	53a	46a	26a
Random Gen.	35a	32a	29a	54a	58a	54a	1.4a	1.7a	1.4a	4.4a	6.1a	4.6a	0.44a	12a	12b	9b	55a	19a	17a	14a	60a	51a	44a	22a
Wild European Pop.	36a	32a	31a	52a	57a	53a	1.5a	1.8a	1.4a	4.5a	6.1a	4.6a	0.48a	12a	13a	9a	53a	19a	17a	14a	60a	50a	44a	22a
Wild Asian Pop.	36a	32a	30a	51b	56a	51b	1.4b	1.6b	1.4a	4.5a	6.1a	4.5a	0.43a	11b	11b	8b	57a	21a	18a	15a	58a	48b	40b	17b
Falcata Clones																								
Top 20 Yielding Gen.	37**	32^{NS}	31**	39^{NS}	47^{NS}	40^{NS}	1.6^{NS}	2.0^{NS}	1.9***	4.1^{NS}	5.8^{NS}	4.2^{NS}	0.25^{NS}	9^{NS}	10^{NS}	7^{NS}	56a	31a	16a	14a	42^{NS}	36^{NS}	29^{NS}	12^{NS}
Selected Gen.	35a	31a	29a	40a	49a	44a	1.5a	1.9a	1.7a	4.2a	5.9a	4.3a	0.25a	9a	10a	7a	56a	26a	17a	13a	42a	39a	31a	13a
Random Gen.	33a	30a	27a	37a	47a	40a	1.5a	2.0a	1.7a	4.0a	5.8a	4.3a	0.20a	8a	9a	7a	61a	34a	19a	12a	39a	34a	27a	11a
Wild European Pop.	34a	31a	29a	35a	44a	37a	1.5a	2.0a	1.8a	4.2a	6.0a	4.3a	0.22a	8a	9a	7a	63	38a	15a	12a	36a	31a	25a	10a
Wild Asian Pop.	32a	31a	26b	27b	38b	32b	1.3a	1.8a	1.4b	4.1a	6.0a	4.2a	0.17a	8a	8b	6b	–	52a	13a	10a	27b	25b	17b	8b

*,**,*** significant at $p = 0.05$, 0.01, and 0.001 levels respectively. ^{NS} not significant.
† Spring regrowth.
‡ Autumn height (33–52 days after 3rd harvest plant height average).
§ Indicated differences significant at $p = 0.05$.
¶ Mean deviation of sativa × falcata crosses from the average of sativa × sativa crosses and falcata clones.

vigor. The wild European SFC had superior first and third harvest growth habit; increased first and second harvest vegetative density; faster second, third, and fourth growth period regrowth; and increased second and third harvest and autumn height compared to SFC from wild Asian genotypes (Table 2). The agronomic superiority of the European germplasm for morphological traits mirrors the results for biomass yield (Riday & Brummer, 2003).

Falcata clones

The falcata parental clones of the top 20% yielding SFC, compared to the remaining clones, had greater first and third harvest plant width and third harvest vegetative density, but no other differences were noted (Table 2). Within the same population, selected and unselected falcata genotypes did not differ for any of the traits. Falcata genotypes from wild European populations had greater third harvest plant width and vegetative density, more erect growth habit and greater plant height during all three harvests, and faster regrowth during third and fourth growth periods than falcata from wild Asian populations.

Growth curves

A Gompertz curve was fitted through height series data for SSC, SFC, SC, and FC (Figure 3). The SC curve was very similar to the SSC curve and was omitted from the figures to improve presentational clarity. A mid SSC-FC curve was also included as the average of the SSC and FC curves. Throughout the growing season, SSC were taller than SFC, which in turn were taller than FC, as expected based on Table 2 and our previous work (Riday and Brummer, 2002b). Sativa-falcata hybrids have slower regrowth than expected under an additive model; however, they have greater height at harvest than expected (Riday and Brummer, 2002b, Table 2). Using the height series information, we attempted to estimate the number of days after harvest at which sativa-falcata hybrid yield switches from being inferior to being superior to the mid-subspecies mean. To accomplish this comparison, we assumed that biomass accumulation mimics the trajectory of plant height over time. Based on this assumption, we see that SFC begin showing superior yield to SSC at about the beginning of May and continuing until the time of first harvest (Figure 4). Following first harvest, the SFC regrow slower than the SSC; however, as the SSC begin to reach their asymptote, the SFC narrow the difference between them. The inferior yield of SFC to SSC, particularly during the early part of the growth period, is very pronounced. Thus, SFC produced from current germplasm would not be amenable to a very intense harvesting regime, such as four to five harvests per year, as currently pursued by high producing dairy herds in Iowa and surrounding states. At about 23 days after harvest, SFC go from being inferior to the mid-subspecies curve to being superior (Figure 4). Following second harvest, SFC remained inferior to SSC throughout the growth period; however, less lag in regrowth was evident compared to the second growth period. The SFC curve is inferior to the mid-subspecies curve until about three weeks after the previous harvest.

In this experiment, we attempted to assess the potency of a broad range of falcata germplasm to produce heterosis with elite sativa genotypes and as such, we knew we were including agronomically undesirable falcata germplasm. Thus, examining the top 20% of the hybrids provides a more realistic view of the current potential of sativa-falcata hybrids (Figure 4). During the first growth period, the yield superiority of the best SFC compared to SSC increases over time. During the second growth period, the top yielding hybrids accumulate biomass slower than the SSC. However, after 28 days, the hybrids begin outyielding SSC because the hybrids continue biomass accumulation as the SSC level off. During third growth period, top yielding SFC and SSC have similar biomass accumulation until about 20 days after harvest, after which the top hybrids accumulate biomass at a faster rate than the SSC.

Population means

The testcross progeny means for individual falcata accessions show substantial variation (Table 3). For plant width, vegetative density, and maturity, variation among falcata populations overlaps that of sativa populations, but for growth angle, regrowth, and plant height, very little overlap exists between falcata and sativa populations. These same traits showed large ratios of variances between populations to within populations variances. Smaller ratios suggest sampling more broadly within populations for such traits may be advantageous. Particularly disappointing from a breeding perspective is the lack of regrowth rates in any falcata populations comparable to sativa. However, a substantial variation exists within falcata, and assuming most of these traits are quantitatively inherited with the potential for transgressive segreg-

Table 3. Testcross progeny population averages for traits measured at: (i) three harvest – plant width, plant growth angle, vegetative density, and maturity score; and (ii) four time periods – regrowth, maximum growth, and plant height at harvest. Reported means are based on two Iowa locations during 2001 and 2002

Population	Plant width			Growth Ang.			Veg. Density			Maturity			Regrowth				Maximum growth				Plant height			
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	S.	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	A.
	—- cm —-			— deg° —			- mg/cm^{-3} -			—- score —-			—- cm —-				— day —				— cm —			
PI 631591	33	29	27	55	58	56	1.3	1.8	1.4	4.6	6.1	4.6	0.37	14	13	10	56	16	15	15	60	46	42	24
PI 251836	29	26	23	62	67	63	1.4	1.7	1.4	4.3	6.0	4.5	0.73	18	15	19	51	14	17	12	62	54	47	48
PI 631579	39	33	32	52	58	55	1.4	1.7	1.4	4.4	6.2	4.7	0.42	14	15	11	49	19	16	13	61	53	48	31
PI 253451	30	27	25	59	65	60	1.4	1.7	1.4	4.6	6.3	4.8	1.21	14	14	13	.	20	18	12	64	55	49	29
PI 631796	35	31	31	55	61	55	1.5	1.8	1.5	4.4	6.1	4.5	0.39	14	14	10	53	19	17	15	64	55	47	24
5291/88	34	31	29	56	61	57	1.7	2.0	1.7	4.5	6.2	4.7	0.53	15	15	13	62	18	16	12	63	54	47	29
5299/88	34	31	29	55	60	56	1.5	1.7	1.5	4.5	6.2	4.7	0.60	14	14	14	63	22	18	11	62	54	47	33
PI 631857	41	35	34	51	55	50	1.3	1.7	1.3	4.4	5.9	4.6	0.58	10	12	8	56	23	21	16	65	51	46	19
PI 494661	36	33	33	49	54	50	1.6	2.0	1.6	4.6	6.2	4.8	0.48	11	12	9	50	19	18	16	54	46	41	22
PI 494658	30	27	25	61	67	62	1.6	1.9	1.6	4.7	6.4	4.9	0.96	19	16	18	57	14	15	12	67	58	52	43
PI 502441	36	32	28	54	59	56	1.4	1.7	1.4	4.4	6.0	4.7	0.47	11	12	9	55	21	21	16	62	53	47	21
PI 384890	43	40	37	38	44	39	1.4	1.7	1.4	4.4	6.1	4.6	0.69	10	9	9	.	24	20	14	54	46	39	20
PI 538985	39	34	32	49	54	48	1.4	1.7	1.4	4.6	6.1	4.6	0.47	10	11	8	52	21	17	15	57	47	38	16
PI 440539	34	31	26	54	60	57	1.1	1.3	1.1	4.2	6.2	4.9	0.29	12	11	7	56	22	21	18	61	52	44	19
PI 631608	32	29	26	53	57	55	1.5	1.7	1.4	4.7	6.2	4.9	0.26	12	12	9	53	19	21	15	55	45	41	20
PI 631612	35	30	29	53	58	55	1.1	1.4	1.1	4.7	6.3	5.0	0.39	12	12	9	56	20	20	15	60	49	45	22
PI 631601	35	31	28	50	57	53	1.3	1.6	1.3	4.4	6.2	4.6	0.36	11	11	8	60	19	18	15	55	47	40	19
PI 538993	38	33	31	50	55	49	1.4	1.7	1.5	4.6	6.2	4.3	0.55	10	11	8	57	20	18	15	57	48	38	15
PI 577561	37	34	30	49	52	47	1.5	1.7	1.6	4.5	6.1	4.1	0.54	11	11	8	57	20	15	14	53	44	34	16
PI 499661	37	33	31	52	54	49	1.4	1.6	1.3	4.5	6.0	4.7	0.58	11	11	8	54	20	18	17	59	48	39	18
PI 631645	40	36	33	47	53	46	1.4	1.7	1.5	4.8	6.3	4.7	0.43	10	10	8	54	23	18	16	56	48	36	14
PI 631639	37	33	32	52	57	51	1.2	1.6	1.3	4.4	6.1	4.5	0.48	11	12	8	58	20	17	16	60	51	42	18
PI 499548	35	33	31	54	58	54	1.3	1.5	1.3	4.1	5.8	4.2	0.39	12	12	8	58	22	20	17	64	54	45	18
W6 16608	34	31	31	53	57	48	1.1	1.5	1.3	4.1	5.8	4.3	0.48	10	10	7	64	24	20	14	63	49	38	13
PI 214218	37	31	29	55	62	56	1.4	1.6	1.4	4.5	6.0	4.6	0.31	12	13	9	53	20	17	16	65	56	46	23
PI 631806	36	34	30	50	54	49	1.6	1.8	1.5	4.8	6.4	4.6	0.42	12	12	9	53	18	16	13	55	47	37	17
PI 314092	34	32	29	55	58	55	1.4	1.6	1.3	4.2	6.0	4.5	0.45	13	13	9	49	18	16	14	59	52	44	23
PI 631811	36	30	28	54	61	57	1.2	1.4	1.1	4.2	5.4	4.3	0.31	13	13	8	63	19	21	15	65	54	50	17
PI 573175	35	33	31	54	57	50	1.5	1.6	1.4	4.2	5.9	4.3	0.41	11	12	8	56	20	17	13	62	50	40	18
PI 631620	34	30	28	55	61	57	1.3	1.5	1.2	4.3	6.1	4.5	0.39	13	13	9	54	18	19	15	63	54	47	23
PI 631597	30	28	25	58	62	59	1.4	1.6	1.4	4.2	6.0	4.5	0.42	14	14	11	59	17	17	14	59	53	46	29
PI 631596	32	32	28	58	59	57	1.5	1.6	1.3	4.4	6.0	4.6	0.40	14	13	9	56	18	18	15	64	54	48	24
PI 502453	34	32	30	56	59	55	1.5	1.7	1.4	4.1	5.8	4.4	0.43	12	13	9	54	20	18	14	64	54	45	25
PI 631797	34	31	29	54	58	54	1.4	1.6	1.3	4.4	6.2	4.5	0.39	13	13	9	53	17	16	14	58	50	43	23
PI 631799	33	29	25	57	61	59	1.4	1.5	1.4	4.7	6.4	4.8	0.33	15	14	11	60	16	16	15	63	53	46	29
IA-3018	33	31	28	58	63	61	1.4	1.6	1.4	4.4	6.2	4.7	0.34	16	16	12	53	17	18	14	66	60	55	35
Lodgeland	39	34	32	51	56	51	1.5	1.7	1.5	4.3	5.8	4.3	0.39	12	12	8	56	19	16	13	60	51	42	19
SD 201	31	30	29	59	62	57	1.4	1.6	1.3	4.1	5.6	4.6	0.38	11	12	8	55	21	23	15	69	57	51	20
PI 560333	33	29	27	59	63	61	1.4	1.6	1.3	4.3	6.1	4.7	0.36	14	14	11	55	19	20	15	68	57	54	33
PI 468015	33	32	26	57	62	62	1.5	1.6	1.4	4.1	6.1	4.8	0.28	17	15	11	54	16	16	15	62	60	51	31
Pioneer Hi-Bred	30	30	26	61	63	64	1.3	1.6	1.2	4.6	6.4	5.0	0.30	19	19	16	51	13	14	13	67	59	55	46
Forage Genetics	31	31	26	61	63	63	1.3	1.6	1.2	4.5	6.5	5.1	0.32	20	18	16	56	13	14	13	67	59	56	47
Hungarian	28	29	23	62	65	65	1.3	1.5	1.2	4.4	6.4	5.0	0.29	20	20	16	54	13	14	12	65	60	56	47
ABI Alfalfa	30	29	24	63	65	66	1.3	1.5	1.2	4.4	6.3	5.1	0.35	19	18	15	55	14	16	14	72	63	59	46
LSD†	4	4	4	4	4	4	0.3	0.3	0.3	0.4	0.3	0.4	0.19	2	2	2	7	3	2	3	7	5	5	5
B/W Pop. $\sigma^{2‡}$	2	1	1	5	4	6	0.2	0.2	0.4	0.6	1.6	1.2	1.70	9	8	15	–	–	–	–	2	3	5	12

† Approximate least significant difference ($p = 0.05$). ‡ Ratio of between population variance to within population variance.

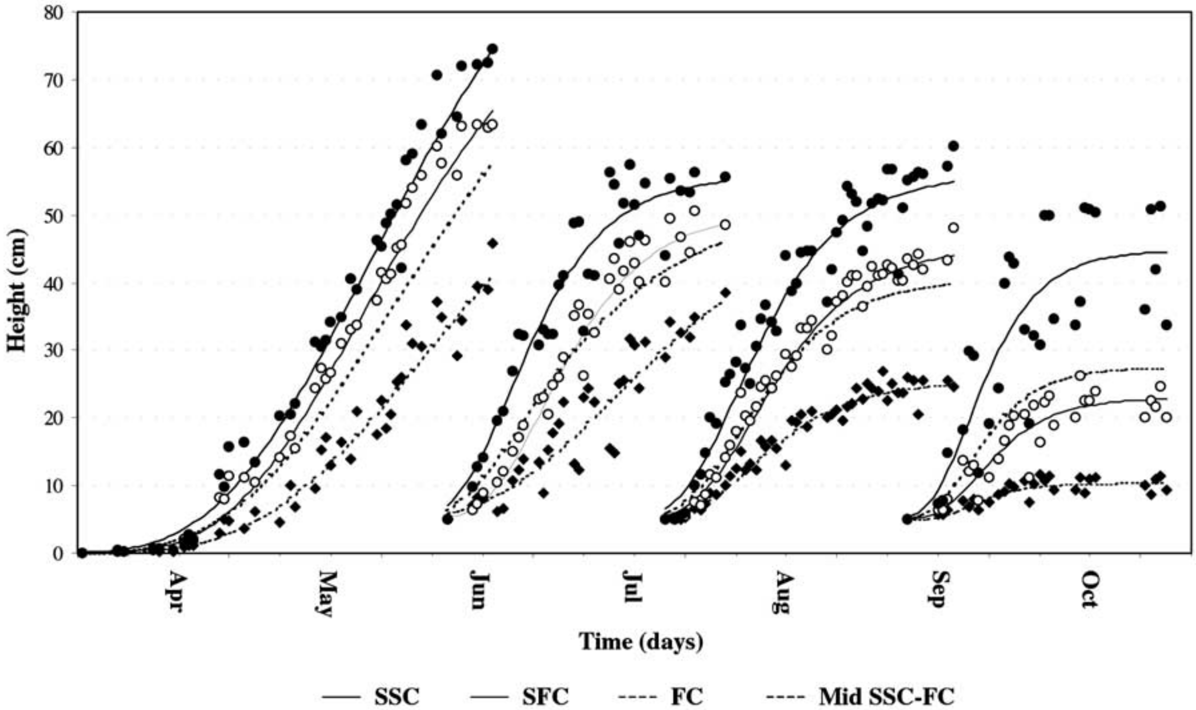

Figure 3. Fitted Gompertz plant height curves based on experiment wide plant heights over time for four growth periods for sativa by sativa crosses (SSC), sativa by falcata crosses (SFC), falcata clones (FC), and estimated mid-subspecies mean (Mid SSC-FC). After first harvest growth curves start at 5cm due to reflect stubble height after cutting.

ation, moving regrowth, height, and growth angle toward sativa may be possible. Falcata populations that had superior agronomic performance in sativa-falcata hybrid combinations included wild populations PI 631796, 5291/88, and 5299/88 and almost all improved falcata populations. Populations PI 631857 and PI 494661 had very high incidence of creeping-rootedness (over 80%). Although these two populations were not particularly impressive *per se*, in hybrid combination with elite sativa germplasm, the populations produced very dense vegetative plots with many stems per 'crown' (due to creeping-rootedness, it was not obvious where one crown ended and the next one began, producing a 'carpet effect'). The creeping-rooted hybrids produced good yield, but did not have desirable height or post harvest regrowth.

Discussion

Heterosis was not only observed for biomass yield but for plant width, growth angle, and plant height. Negative heterosis was observed for regrowth in sativa-falcata hybrids. As expected, sativa germplasm exhibited fast regrowth and an erect growth form. For plant width, plant height, growth angle, density, and maturity, a full range of parental genotypes were found among falcata germplasm. Disappointingly, but not unexpectedly, no falcata germplasm was noted that had regrowth and fall plant height equal to the sativa germplasm level. It is clear that to obtain useful falcata germplasm traits such as plant height and regrowth will need to be improved. It remains unresolved if selection for these traits will adversely affected biomass yield.

Top yielding sativa-falcata hybrids were taller, wider, and denser than the other hybrids. European germplasm, in particular, exhibited increased plant height and regrowth in relation to Asian material, which may help explain why the European falcatas make better hybrids. Only parental plant height, and third harvest plant density had major predictive value for hybrid biomass yield. No parental morphological traits were associated with biomass heterosis *per se*.

Based on height growth rate data, biomass yield of hybrid progeny becomes superior to the mid-subspecies mean about 30 days after the first harvest.

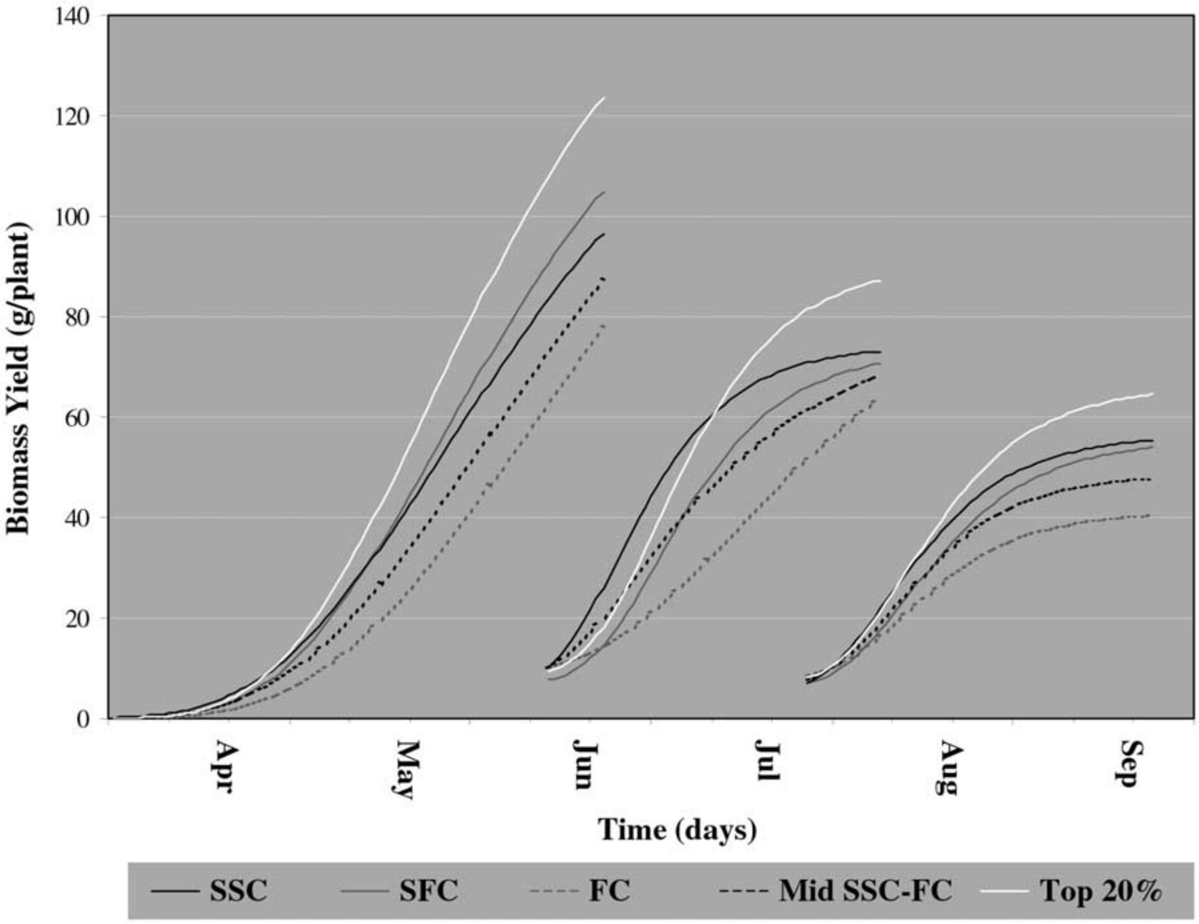

Figure 4. Estimated biomass accumulation over time for sativa crosses (SSC), sativa by falcata crosses (SFC), falcata clones (FC), estimated mid-subspecies mean (Mid SSC-FC), and top 20% yielding SFC (Top 20%). Plant height growth rate information and harvest biomass yields were used to interpolate curves.

During first and third harvest, the sativa-falcata hybrid biomass production is never inferior to and eventually becomes superior to the mid-subspecies expectation.

Although falcata with superior morphology leads to superior sativa-falcata hybrids, the superiority is uncorrelated with biomass heterosis levels. For breeders selecting falcata parents to form a base population, this means that there is no specific morphology that will lead to increased sativa-falcata hybrid heterosis. Selecting falcata parents for good agronomic qualities, however, will increase agronomic performance of sativa-falcata hybrids independent of heterotic performance. Based on this study breeders will be able to make more targeted selections of available falcata germplasm, and should focus in particular on western Eurasian material for adaptation to the Midwestern U.S.A. We have also presented a holistic view, particularly with the growth curve data, of traits that influence biomass yield accumulation over time. Our results will allow targeted selection schemes to optimize stand growth in such a way that optimizes biomass yield while maintaining an economically feasible harvest to harvest interval time. Cultivar development using sativa-falcata hybrids will need to done in concert with the producer's intended management scheme.

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